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Gene Frequencies of Haemoglobin Genotype, ABO and Rhesus Blood Groups among Students Population of a Private University in Nigeria-Implications for Blood Banking

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ABSTRACT

Background- ABO and Rhesus blood groups are the two most important blood group systems of clinical significance. ABO is classified into four major groups (A, B, AB and O), while Rhesus is classified as positive or negative. Haemoglobin genotype included both normal (HbA) and the variant forms (HbS and HbC), which combined to form six major haemoglobin gene types (HbAA, HbAS, HbAC, HbCC, HbSS and HbSC). Several studied have determined the gene frequencies of haemoglobin genotype, ABO and Rhesus blood groups among different populations with varied patterns. This study was aimed at assessing the gene frequencies of haemoglobin genotype, ABO and Rhesus blood groups among Redeemer's University students.

Methods- This study was a retrospective study which analyzed laboratory data between 2013 and 2017 containing haemoglobin genotype and Rhesus-ABO investigations carried out for newly admitted students.

Results- The ABO gene frequencies from this study showed O>A>B>AB; 59.7%, 21.6%, 16.6% and 2.0%, ABO with Rhesus combination showed O^+>A^+>B^+>O^->AB^+>A^->B^->AB^- ; 57.1%, 20.8%, 15.9%, 2.6%, 1.8%, 0.8%, 0.7%, and 0.3%. Rhesus positive was found to be far higher than Rhesus negative (Rh^+>Rh^-, 95.6%, 4.4%) and haemoglobin genotype distribution showed HbAA>HbAS>HbAC>HbSS>HbSC>HbCC; 72.5%, 23.4%, 2.4%, 1.5%, 0.2% and 0.1%.

Conclusion- Blood group O, Rhesus positive and haemoglobin AA were found to be more prevalent all through the period under review.

Key-words: ABO, Blood banking, Haemoglobin genotype, Redeemer's University, Rhesus blood group

INTRODUCTION

ABO and Rhesus (Rh) blood groups are usually present on the surface of red blood cells (RBC) and have been found to be the two most important blood group systems of clinical significance, especially, with regards to blood transfusion and organ transplantation [1,2]. The presence or absence of the ABH surface antigens on individual's RBCs determines the particular blood group system [3], the ABH antigens are oligosaccharides in nature and the H antigen is a precursor substance that is converted to either A or B antigen (or both) by specific glycosyltranferases encoded by the ABO gene located on chromosome 9 [4,5].

The ABO gene displays several single nucleotide polymorphisms (SNPs) and has three allelic forms (A, B and O) [6], which has been classified into four major groups (A, B, AB and O) based on the presence or absence of antigens on the red cells and antibodies in the serum [7,8]. Whereas the A and B genes are dominant, the O gene is recessive and expressed only in the absence of the dominant genes, the expression of blood group system is determined by inheritance and not influenced by environmental and other factors except in

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cases of bone marrow transplant and some disease conditions [3,9].

Among the several Rhesus (Rh) antigens, the Rh-D antigen has been found to be the most immunogenic and one of the most complex blood group systems in humans, its clinical significance in blood transfusion is second only to the ABO system. Individuals who lack the Rh-D antigen produce anti-Rh-D antibodies when they encounter the D-antigen on transfused red blood cells (RBC), Rh-D incompatibility could result in serious haemolytic transfusion reaction (HTR) and hemolytic disease of the newborn (HDN) [3,10]

The importance of the ABO and Rh blood group systems in transfusion science cannot be over emphasized as the major components of grouping and cross-matching is to ensure compatibility of the ABO and Rh blood group to avoid transfusion of incompatible blood which can result in transfusion reactions. Hence, in transfusion science, every blood must be screened for ABO and Rh compatibilities before transfusion [11,12]. Furthermore, it has also been reported that the blood group systems play significant roles in various human diseases such as diabetes, cardiovascular diseases, neoplasm, carcinoma and some infectious diseases. These roles make the ABO and Rh blood group systems of great importance in modern medicine [11,12]

Although the ABO and Rh are the most clinically relevant blood group systems, their prevalence varies from tribe to tribe [13] and place to place [14]. Hence, the knowledge of their distribution is essential for smooth running of blood banks [15]. Particularly, the type and stock levels of blood and blood products available in a blood bank should be proportional to the distributions of the ABO and Rh in the general population of the community the blood bank serves [10].

The haemoglobin (Hb) molecule comprises two basic units, the globin and the haem prosthetic group. Structurally, Hb is tetrameric and consists of two different pairs of globin chains, each attached to one haem molecule. The globin functions as the proteinous part, while the haem prosthetic group serves as the oxygen-carrying component of the molecule [16]. Hb genotypes include both normal (HbA) and variant/ abnormal forms (HbS and HbC), the variant HbC is formed by the replacement of glutamic acid with lysine at the 6th position of the β-globin chain of the molecule and causes a mild chronic hemolytic anaemia.

The inheritance of HbC from both parents results in a homozygous state (HbCC), HbC can also combine with HbA and HbS at the point of inheritance to form HbAC and HbSC respectively [9,17,18]. HbS has valine replacing glutamic acid at the 6th position of the β-globin chain of the molecule. The inheritance of HbS from both parents results in a homozygous state (HbSS) known as sickle cell anaemia/disease (SCA/SCD), whereas the inheritance of HbS from one parent and HbA from the other leads to a heterozygous state (HbAS) which is known as sickle cell trait (SCT). The clinical features of HbSS include haemolytic anaemia, jaundice, fever, joint ache, skeletal changes due to erythroid hyperplasia, painful infarcts, pulmonary complications, kidney damage, haemolytic, and aplastic anaemia among others and they often require specialized medical care and other forms of support [9,18]. There are six major haemoglobin gene types inherited in the homozygous (HbAA, HbCC, and HbSS) or heterozygous state (HbAS, HbAC, and HbSC) [17].

The knowledge of the gene frequencies of Hb genotype, ABO, and Rh blood groups in a population is required for adequate healthcare planning and policy formulation. Several studied, Igbeneghu *et al*. [2]; Omotosho [16]; Medugu *et al*. [18] determined the gene frequencies of haemoglobin genotype, ABO and Rh blood groups have been conducted with varied patterns of distribution among different populations and ethnic groups all over the world. This study was aimed at assessing the gene frequencies of haemoglobin genotype, ABO, and Rhesus blood groups among students population and its implications for blood banking.

MATERIALS AND METHODS

This study was a retrospective cross-sectional study which involved analyzing laboratory data for a period of five years (2013–2017), the University's Institutional Research Ethics Committee approved the study protocol (RUN-IREC: 009). Laboratory database containing Hb genotype and Rhesus ABO investigations carried out for newly admitted students at the Redeemer's University Health Centre between 2013 and 2017 (5 years) was assessed for the study. To ensure patient confidentiality, data collected from the laboratory database were de-identified and only authorized personnel was allowed to access the database. As a result of the diversity in the distribution of blood types, all the students screened for haemoglobin genotypes, ABO and Rhesus blood groups

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for the five years period under review were included in the study. The data was systematically collated, analyzed and presented according to blood type and year.

RESULTS

A total of 1900 participants were eligible for the study comprising 1069 (56.3%) females and 831 (43.7%) males with a mean age of 19.5 years. The results (Table 1) showed that blood group O had the highest frequency (59.7%) followed by blood groups A, B, and AB with 21.6%, 16.6% and 2.0% respectively. The ABO gene frequencies (Table 1) from our study showed O>A>B>AB pattern, while ABO with Rhesus combination (Table 2) gave the following pattern: O^+>A^+>B^+>O^->AB^+>A^->B^->AB^- with values 57.1%, 20.8%, 15.9%, 2.6%, 1.8%, 0.8%, 0.7%, and 0.3% respectively.

All through the five years period of this study, group O maintained the highest gene frequency followed by groups A and B, except in 2015 where group B was slightly higher than group A, blood group AB had the lowest gene frequency all through the period under review (Table 1). The gene frequency of Rh blood group (Table 1) shows Rh^+ far higher than Rh^- (Rh^+>Rh^-: 95.6%, 4.4%). O^+ showed the highest frequency distribution all through the period under review followed by A^+ except in 2015 where B^+ was slightly higher than A^+ while O^- was seen having higher gene frequency than AB^+ all through the period of study (Table 2).

Table 1: Percentage (%) gene frequencies of ABO and Rh blood groups

YEAR	O	A	B	AB	Rh^+	Rh^-
2017	57	24.1	17.5	1.4	96.0	4.0
2016	59.1	22.1	16.2	2.6	96.0	4.0
2015	52.6	21.7	22.8	2.9	95.0	5.0
2014	62.6	21.6	14.8	1.0	95.0	5.0
2013	67.4	18.7	11.6	2.3	96.0	4.0
MEAN	59.7	21.6	16.6	2.0	95.6	4.4

Table 2: Percentage (%) gene frequencies of ABO-Rh blood groups

YEAR	O^+	A^+	B^+	AB^+	O^-	A^-	B^-	AB^-
2017	55.1	23.6	16.0	1.4	1.9	0.5	1.5	–
2016	56.8	21.5	15.6	2.3	2.3	0.6	0.6	0.3
2015	50.0	20.2	22.4	2.2	2.6	1.5	0.4	0.7
2014	58.8	20.6	14.5	1.0	3.8	1.0	0.3	–
2013	64.9	18.1	10.8	1.9	2.5	0.6	0.8	0.4
MEAN	57.1	20.8	15.9	1.8	2.6	0.8	0.7	0.3

The gene frequency of Hb genotype in our study showed that HbAA had the highest (72.5%) frequency and HbCC the lowest (0.1%). The pattern of Hb genotype distribution in our study can be summarized as HbAA>HbAS>HbAC>HbSS>HbSC>HbCC: 72.5%, 23.4%, 2.4%, 1.5%, 0.2% and 0.1% (Table 3).

Table 3: Percentage (%) gene frequencies of Hb genotype

YEAR	HbAA	HbAS	HbAC	HbSS	HbSC	HbCC
2017	72.7	23.1	2.7	1.0	0.5	–
2016	71.9	23.3	2.0	2.2	0.3	0.3
2015	68.7	27.3	2.9	1.1	–	–
2014	74.1	21.7	2.6	1.6	–	–
2013	75.2	21.4	1.6	1.4	0.4	–
MEAN	72.5	23.4	2.4	1.5	0.2	0.1

DISCUSSION

This study was carried out to determine the gene frequencies of Hb genotype, ABO and Rhesus blood groups among students population and its implications for blood banking. From this study, blood group O was the most predominant group, occurring in three-fifth of the study participants. The pattern (O>A>B>AB) of ABO gene frequencies in our study is consistent with previous studies, Igbeneghu *et al*. [2]; Anifowoshe *et al*. [3]; Medugu *et al*. [18]; Adeyemo and Soboyejo [19]; Enosolease and Bazuaye [20]; Faduyile *et al*. [21]; Odegbemi *et al*. [22] carried out in different parts of Nigeria. A study conducted in Lagos, Nigeria, Odegbemi *et al.* [22] reported the prevalence of groups O, A, B and AB as 51.8%, 26.3%, 18.2% and 3.6% respectively. One nation-wide study, Anifowoshe *et al.* [3] reported similar pattern of ABO distribution (O>A>B>AB: 52.93%, 22.77%, 20.64% and 3.66% respectively). Likewise, similar studies conducted among African students in Port Harcourt [23], Niger Delta area [24] and Benin City area of South-South, Nigeria [20] reported values similar to our findings.

However, other similar studied, Onuoha *et al*. [8]; Erhabor *et al*. [10]; Etim *et al*. [13]; Omotosho [16]; Akhigbe *et al*. [17]; Muhibi *et al*. [25] didn't agree with the pattern of ABO distribution in our study. Although in all these studied, group O and AB had the highest and lowest prevalence respectively, which was in tandem with our findings. In one of the studied conducted in Muhibi *et al.* [25], the authors found group B (21.3%) slightly higher than group A (21.1%) in contrast to our study which could be attributable to the study populations (blood donors) of the study and the variable nature of the ABO blood group system. Also, Etim *et al*. [13] study conducted in Adamawa, North East, Nigeria found blood group gene frequencies to be 56.2%, 21.3%, 17.7% and 4.7% for O, B, A, and AB, respectively. Likewise, in another studied Onuoha *et al*. [8]; Erhabor *et al*. [10]; Akhigbe *et al*. [17], group B was found to be higher than group A.

Beyond Nigeria, our findings agreed with the findings of other investigators who reported similar patterns of gene frequencies for ABO blood groups in Uganda [14], Ethiopia [26], Tanzania [27], Saudi Arabia [28] and Brazil [29] respectively. Our findings differ from one Pakistani study [30] and another Indian study Agrawal *et al.* [15]. However, one study in Nepal, Pramanik and Pramanik [31] reported group A as the most prevalent gene frequency while another study in Pakistan, Hameed *et al*. [32] reported group B as the most prevalent.

The distribution of Rh blood group is also known to vary among different populations, nevertheless, Rh^+ has been found to be highly predominant compared to Rh^-. The pattern observed in our study (Rh^+>Rh^-: 95.6>4.4%) was generally consistent with previous studied Anifowoshe *et al*. [3]; Etim *et al*. [13]; Omotosho [16]; Akhigbe *et al*. [17]; Medugu *et al*. [18]; Adeyemo and Soboyejo [19]; Enosolease and Bazuaye [20]; Faduyile *et al*. [21]; Odegbemi *et al*. [22] within Nigeria, while we reported a value of 4.4% prevalence for Rh^- in our study, another studied reported values as low as 2.9% in Yola, Nigeria [18], 2.3% in Uganda [14] and 1.2% in Gusau, Nigeria [10].

The low gene frequency of Rh^- blood group reported in this study had advantaged in blood banking and disease management (HDN) [14]. With regards to blood banking, it presents a reduced demand for Rh^- blood for transfusion purposes as such demands usually poses a herculean task to blood bank managers. It also confers some obstetric advantages on the population with regards to

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Rh alloimmunization and attendant HDN which often occur when a Rh^- mother becomes pregnant with a Rh^+ child (inherited from the Rh^+ father) [10]. However, it was well noted that Rh alloimmunization accounts significantly for perinatal morbidity in most resource-limited countries [10]. Furthermore, the occurrence of different Rh blood groups in the study population calls for pre-marital counseling advocacy, which will enable Rh^- females who would marry Rh^+ males take preventive measures against fetal loss and infant mortality due to HDN.

HbAA maintained the highest distribution all through the period under review, followed by HbAS, HbAC and HbSS except in 2016, where HbSS was slightly higher than HbAC. Generally, the pattern of Hb genotype distribution observed in our study was similar to previous studied Akhigbe *et al*. [17]; Medugu *et al*. [18]; Adeyemo and Soboyejo [19] in Nigeria. In our study, we observed the incidences of HbAC (2.4%), HbSC (0.2%) and HbCC (0.1%) as consistent with the findings of a similar study among the Yoruba's in Ibadan [33] and another study among the Ika ethnic group of Delta State [34]. The low value (0.1%) reported for HbCC in our study was consistent with the findings of previous investigators in Nigeria, Omotosho [16]; Akhigbe *et al*. [17], who reported 0.18% and 0.2% respectively, while another investigator [19] reported as high as 0.7%. Meanwhile, other investigators Igbeneghu *et al*. [2]; Onuoha *et al*. [8]; Medugu *et al*. [18]; Umoh *et al*. [35] had no report of HbCC in their study. The difference could be due to the variable nature of the haemoglobin variant.

HbC is one of the most common structural haemoglobin variants in the human population, HbC trait (HbAC) is asymptomatic and such heterozygote individuals are phenotypically normal, while homozygote (HbCC) persons that is HbC disease may have mild degree of haemolytic anaemia due to the reduced solubility of RBCs which can lead to crystal formation, splenomegaly and border-line anaemia [36]. However, when this HbC variant is inherited along with HbS, that is, sickle-HbC disease (HbSC), significant clinical consequences such as chronic haemolytic anaemia and occasional sickle cell crises may occur. Although the prevalence of HbC trait in our present study was low (2.4%), there is still the need for haemoglobin genotype screening for potential couples in order to keep HbSC and HbCC out of the population.

The SCD prevalence depends on the prevalence of SCT in the general population, and where the prevalence of SCT exceeds 20%, SCD was estimated to be at least 2% [37]. Although in our present study we found the proportion of those with SCT to be 23.4%, the prevalence of SCD was only 1.5%. These findings suggest that SCD was on the decline as had been observed in one study [17], which could be attributable to parental Hb genotype testing prior to marriage, improved pre-marital counseling; awareness of the dangers of sickle cell anaemia improved socio-economic status and increased awareness of fetal Hb genotype screening.

CONCLUSIONS

Our findings on the gene frequencies of the two clinically most important blood group systems have great implications for blood banking as it calls for stringent pre-transfusion compatibility tests to prevent haemolytic transfusion reactions associated with ABO and Rh incompatibilities. While it is generally good to have a large stock of the more prevalent blood groups in the blood bank, adequate effort should be made to also have the rare blood groups such as A^-, B^-, and AB^- readily available due to the slim chances of recruiting such donors in emergency situations. Likewise, in view of the high prevalence of SCT in our study, continued Hb genotype test and premarital counseling of potential couples is advocated including sustainable SCD surveillance in view of achieving the WHO vision 2020 for curbing the menace of SCD. Blood group O, Rh^+, and HbAA were found to be more prevalent all through the period under review.

Operational blood banks should be equipped with facilities to screen for Hb genotype, ABO, and Rh blood groups in order to effectively meet up with the demand for safe blood transfusion. In addition, we look forward to the availability and use of simpler, quicker and more informative molecular methods for the determination of Hb genotype, ABO, and Rh blood groups.

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